

Amendments to the Specification:

Insert the paper copy of the Sequence Listing filed herewith following the Abstract.

Please amend the paragraph beginning at page 1, line 20, as follows:

The amino acid sequence of the wild type *Fibrobacter succinogenes* 1,3-1,4- $\beta$ -D-glucanase (SEQ ID NO: 1) and the nucleotide sequence (SEQ ID NO: 6) encoding it are listed below:

ATGAACATCAAGAAAATGCAGTCAGAGCGCTCTGCCGTAGCAGCCGCAGCAGCAGCC  
M N I K K T A V K S A L A V A A A A A A A 20

CTCACCAACATGTTAGCGCAAAGGATTTAGCGGTGCCAACTCTACACGTTAGAAGAA  
L T T N V S A K D F S G A E L Y T L E E 40

GTTCACTACGGTAAGTTGAAGCCCGTATGAAGATGGCAGCCGCATGGAACAGTCAGT  
V Q Y G K F E A R M K M A A A S G T V S 60

TCCATGTTCCCTTACCAAGAATGGTCCGAAATGCCGATGGAAGGCCCTGGGTAGAAGTG  
S M F L Y Q N G S E I A D G R P W V E V 80

GATATTGAAGTTCTCGGCAAGAATCCGGGAGTTCCAGTCCAACATCATTACCGTAAG  
D I E V L G K N P G S F Q S N I I T G K 100

GCCGGCGCACAAAAGACTAGCGAAAAGCACCATGCTGTTAGCCCCGCCGATCAGGCT  
A G A Q K T S E K H H A V S P A A D Q A 120

TTCCACACCTACGGTCTCGAATGGACTCCGAATTACGTCCGCTGGACTGTTGACGGTCAG  
F H T Y G L E W T P N Y V R W T V D G Q 140

GAAGTCCGCAAGACGGAAGGTGGCCAGGTTCCAACCTGACAGGTACACAGGGACTCCGT  
E V R K T E G G Q V S N L T G T Q G L R 160

TTAACCTTGGTCGTCTGAGAGTGCAGGTTGGCCAGTTGATGAATCAAAGCTT  
F N L W S S E S A A W V G Q F D E S K L 180

CCGCTTTCCAGTTCATCAACTGGGTCAAGGTTATAAGTATACGCCGGCCAGGGCGAA  
P L F Q F I N W V K V Y K Y T P G Q G E 200

GGCGGCAGCGACTTACGCTTGACTGGACCGACAATTTGACACGTTGATGGCTCCCGC  
G G S D F T L D W T D N F D T F D G S R 220

TGGGGCAAGGGTGACTGGACATTTGACGGTAACCGTGTGACCTCACCGACAAGAACATC  
W G K G D W T F D G N R V D L T D K N I 240

TACTCCAGAGATGGCATGTTGATCCTCGCCCTACCCGCAAAGGTCAAGAAAGCTTCAAC  
Y S R D G M L I L A L T R K G Q E S F N 260

GGCCAGGGTCCGAGAGATGACGAACCTGCTCCGAAATCTCTAGCAGCGCTCCGGCATCT  
G Q V P R D D E P A P Q S S S S A P A S 280

TCTAGCAGTGTCCGGCAAGCTCCTCTAGCGTCCCTGCCTCCTCGAGCAGCGCATTGTT  
S S S V P A S S S S V P A S S S S A F V 300

CCGCCGAGCTCTCGAGCGCCACAAACGCAATCCACGGAATGCGCACAACTCCGGCAGTT  
P P S S S S A T N A I H G M R T T P A V 320

GCAAAGGAACACCGCAATCTCGTAACGCCAAGGGTGCCAAGGTGAACCGAATGGCCAC  
A K E H R N L V N A K G A K V N P N G H 340

AAGCGTTATCGCGTGAACCTTGAAACACTAA  
K R Y R V N F E H \* 349

Please amend the paragraph beginning at page 6, line 10, as follows:

A nucleic acid was amplified from the full-length *Fibrobacter succinogenes* 1,3-1,4- $\beta$ -D-glucanase (Fs $\beta$ -D-glucanase) cDNA (Chen et al. (2001), J. Biol. Chem. 276, 17895-17901) by the PCR using the following two primers: Oligo A: 5'-CAGCCGGCGATGCCATGGTTAGC GCA-3' (SEQ ID NO: 17) and Oligo B: 5'-CTGCTAGAAGAATTGGAGCAGGTTCGTC-3' (SEQ ID NO: 18). The amplified nucleic acid encodes a polypeptide that corresponds to a fragment from aa 24 to 272 of SEQ ID NO: 1, except that the N24 was replaced with M. The polypeptide lacks the C-terminal 78 aa of Fs $\beta$ -D-glucanase. To generate an expression vector, the amplified nucleic acid was digested with Nco I and Eco RI and then ligated into a pET26b(+) vector (Novagen, WI) that had been digested with the same enzymes. The resultant vector was confirmed by DNA sequencing. This construct, designated as pPCR-TF-glucanase, encodes a fusion protein (SEQ ID NO: 10) that has a *pel* B leading peptide sequence (KYLLPTAAAGLLLLAAQPAMA, SEQ ID NO: 11) at the N-terminus and a 19-residue segment (SEQ ID NO: 16) at the C-terminus. Once expressed in a host cell, the *pel* B leading peptide sequence was cleaved to generate a mature fusion truncated glucanase, PCR-TF-glucanase (SEQ ID NO: 9).

Please amend the paragraph beginning at page 6, line 25, as follows:

Another truncated  $\text{F}\beta\text{-D-glucanase}$  (SEQ ID NO: 7), designated as "TF-glucanase," was created using PCR-based site-directed mutagenesis. This TF-glucanase lacks the just-described 19-residue segment at its C-terminus. To make a nucleic acid encoding it, a stop codon was introduced right after the codon for P248 of the just-described pPCR-TF-glucanase. A pair of complementary mutagenic primers were used. The sense strand primer has the sequence: 5'-CCTGCTCCGTAAATCGAGCTCC-3' (SEQ ID NO: 19). The mutagenesis was carried out in a PCR reaction mixture containing 10 mM KCl, 10 mM  $(\text{NH}_4)_2\text{SO}_4$ , 20 mM Tris-HCl (pH 8.8), 2 mM  $\text{MgCl}_2$ , 0.1% Triton<sup>R</sup> X-100, 0.1 mg/ml nuclease-free BSA, 10-15 ng of template DNA, 0.2 mM dNTPs, 0.25  $\mu\text{M}$  each of the primers, and 2.5 units of Turbo *Pfu* DNA polymerase (Stratagene, La Jolla, CA). The PCR reactions were conducted on a Hybaid TouchDown thermal cycler using the following program: 2 min at 95°C, 16 cycles of 1 min at 55°C/13 min at 68 °C/45 sec at 95 °C. The products were digested with 10 units of Dpn I at 37 °C for 1 hour (h) and subsequently transformed into *E. coli* XL-1 Blue competent cells by electroporation. The transformed cells were grown on LB agar plates containing 30  $\mu\text{g}/\text{ml}$  kanamycin at 37 °C until colonies appeared on the plates. The colonies were selected randomly and cultured in 5 ml LB/kanamycin liquid culture at 37 °C for 16 h before plasmids were isolated from the culture using a QIAprep Spin Miniprep kit (Qiagene, Hilden, Germany). Mutation was confirmed by DNA sequencing. The plasmid thus obtained was named "pTF-glucanase."

Please amend the paragraph beginning at page 7, line 13, as follows:

A vector encoding a truncated glucanase having a Trp203→Phe (W203F) point mutation was generated by PCR based site-directed mutagenesis using the above described pPCR-TF-glucanase as the template in the same manner described above. A pair of complementary mutagenic primers were used. The sense strand primer was 5'-CTGGGGCAAGGGTGAC TTCACATTGACGGT -3' (SEQ ID NO: 20). The vector was augmented and prepared from *E. coli* XL-1 Blue, and confirmed by DNA sequencing. The vector and the polypeptide it encodes were designated as pPCR-TF-W203F and PCR-TF-W203F, respectively.

Please amend the paragraph beginning at page 7, line 21, as follows:

A *Pichia* expression vector that encodes a truncated glucanase was generated. Briefly, PCR was used to amplify the DNA sequence encoding V25 to P271 of SEQ ID NO: 1 from pPCR-TF-glucanase. The primers used were listed below:

Oligo C 5'-TACGCTGCAGTTAGCGCAAAGGATTTAGC-3' (SEQ ID NO: 21) and

Oligo D 5'-TAGTTCTA GATCACGGAGCAGGTTCGTCATCTCTC-3' (SEQ ID NO: 22).